Supplementary Material for:

Habitat patchiness drives spatial structure in morphological trait variation and co-variation in spotted salamanders (*Ambystoma maculatum*)

Effect of salamander feeding on mass

All salamanders were fed one tree frog tadpole the day prior to being weighed, but not all salamanders ate the frogs. Acknowledging that differential feeding may influence our mass measurements, we statistically tested whether mass of salamanders that completely consumed tadpoles differed from salamanders that did not. Specifically, we ran generalized linear models with mass as the response and feeding outcome as a categorical fixed effect (consumed or not consumed). Models had a Gaussian error structure because mass values were normally distributed (Fig. 3 in main text). We tested the influence of feeding outcome on mass by comparing a model with feeding outcome as a fixed effect with a model omitting the effect, using a likelihood ratio test. We used a random subset of 237 salamanders for which we had feeding records. Including feeding outcome in the model did not significantly improve model fit ($F_{(1\text{-}82)} = 3.43$, $p = 0.068$). Thus, we did not include feeding outcome as a factor in models used in the main analyses.

Preliminary analysis of pond age and predator density as factors driving spatial structure of salamander morphology

As an initial exploration into potential drivers of spatial structure in morphological variation, we assessed whether salamander mass, length, mass-length co-variation, and shape were influenced by the age and predator density of ponds. We consider these assessments preliminary because the low replication of ponds in our sample (N = 6).

Methods: We used historical records to classify ponds as 'new' (N = 3) or 'old' (N = 3) (Table S1, below). As we describe in the main text, the three new ponds (Mincke Pond, Arthur Christ Pond, Beth's Pond) were constructed in 2008 for research purposes and had similar sizes and dimensions (Burgett 2015). The three 'old' ponds (Salamander

Pond, Forest 44 Pond, Shaw Pond) had variable construction history. Salamander Pond was created in 1965 while Forest 44 pond and Shaw pond were excavated between 1990 and 1996 (data extracted from Google Earth Historical Imagery).

To estimate predator density, we systematically dip-netted the focal ponds and recorded the abundance and species composition of two broad types of predators of spotted salamander larvae: macro-invertebrates and adult amphibians (Table S5, below). None of the focal ponds contained fish. We were not able to sample Beth's pond for predators, and instead we used historical data collected by EB in 2013. We checked predator density counts in 2013 against our 2016 sampling using Mincke pond, the pond for which we had data from both years. Predator densities in Mincke pond in 2013 were similar to those that we observed in 2016, and so we considered our predator density estimates for Beth's pond to be representative for our sampling period.

To test the influence of pond age and predator density on salamander mass, length, and shape, we ran GLMMs that included pond age ('new' vs. 'old') and predator density as fixed effects, and 'pond' as a random effect. To test whether the age and predator density of ponds influenced mass-length co-variation, we ran GLMMs with log-transformed mass as the response and log-transformed length, the focal factor - pond age or predator density – and their interaction as fixed effects. We also included length (again, log-transformed) as a random slope term, and 'pond' as a random intercept term. We ran separate GLMMs for the two interactions to prevent model overfitting. We also ran separate GLMMs for our different length measures: head length, body length, tail length, total length. We compared the fit of models including the interaction terms with models omitting the interaction terms, using likelihood ratio tests, to test the influence of pond age and predator density on the relationships between mass and length.

Results: Neither pond age nor predator density influenced salamander mass or any measures of length (Table S4). However, these pond attributes did influence certain mass-length relationships and body shapes (Fig. S4, Table S5). Pond age influenced the scaling of mass with head and tail length (Fig. S4, Table S5). In contrast, predator density influenced mass-body length relationships (Fig S2, Table S2). Both pond age and predator density influenced salamander head shape, but neither influenced body or tail shapes. Pond age also influenced the overall shape of salamanders, but predator density did not (Table S4).

Tables and Figures

Pond Name	sample size	Age	predator Density	Diameter (m)
Forest 44	105	old	3.1	30
Shaw	90	old	1.9	48
Salamander	69	old	0.3	69
Arthur Christ	117	new	11.5	8
Beth’s	30	new	10	8
Mincke	118	new	2	8

Table S1. Pond characteristics. Environmental and ecological variables of the six ponds in east-central Missouri from which salamander larvae were collected.

trait	pond	mean	sd	min	max	CV
mass (g)	Salamander	0.65	0.19	0.25	0.93	29.29
	Shaw	0.46	0.13	0.16	0.78	28.39
	Mincke	0.46	0.17	0.17	0.82	36.65
	Arthur Christ	0.33	0.14	0.09	0.72	42.43
	Forest 44	0.44	0.14	0.18	0.80	31.59
	Beth's	0.59	0.14	0.35	0.85	23.79
head length (mm)	Salamander	7.75	1.23	4.29	9.83	15.88
	Shaw	7.95	0.94	6.02	9.87	11.84
	Mincke	7.77	1.14	4.67	10.19	14.68
	Arthur Christ	6.94	1.23	3.69	9.51	17.78
	Forest 44	7.34	1.07	4.47	9.72	14.57
	Beth's	8.54	1.04	6.08	10.69	12.17
body length (mm)	Salamander	14.45	2.18	9.84	18.22	15.08
	Shaw	12.86	1.78	7.29	17.72	13.81
	Mincke	12.97	2.18	7.29	18.43	16.82
	Arthur Christ	11.94	2.14	6.48	17.35	17.97
	Forest 44	14.00	2.10	9.51	19.46	14.98
	Beth's	16.13	1.79	13.50	19.42	11.12
tail length (mm)	Salamander	22.39	4.14	11.33	29.18	18.47
	Shaw	19.26	2.92	10.40	25.60	15.16
	Mincke	18.56	3.24	11.14	25.90	17.43
	Arthur Christ	16.53	3.69	8.58	25.32	22.35
	Forest 44	19.47	2.86	11.06	27.27	14.70
	Beth's	23.28	2.57	18.00	28.14	11.04
total length (mm)	Salamander	44.59	6.49	26.32	53.94	14.56
	Shaw	40.05	4.64	24.90	49.33	11.58
	Mincke	39.30	5.98	26.53	51.57	15.22
	Arthur Christ	35.41	6.50	21.58	51.45	18.35
	Forest 44	40.81	5.10	26.91	53.21	12.49
	Beth's	47.95	4.57	39.31	57.92	9.54

Table S2: Summary of salamander length (head, body, tail, total) and mass. Summary statistics of 2016 survey data of late-phase larval salamander populations in Missouri. Sd – standard deviation, min = minimum value, max = maximum value, CV = coefficient of variation.

Trait	Pond name	mass regression equation
head length	Forest 44	y = 1.00x -1.24
	Shaw	y = 1.21x -1.44
	Salamander	y = 0.97x -1.07
	Arthur Christ	y = 1.58x -1.84
	Beth's	y = 0.53x -0.74
	Mincke	y = 1.64x -1.82
	Overall	**y = 1.52x -1.71**
body length	Forest 44	y = 1.84x -2.49
	Shaw	y = 1.67x -2.2
	Salamander	y = 1.76x -2.25
	Arthur Christ	y = 2.09x -2.76
	Beth's	y = 1.57x -2.13
	Mincke	y = 1.76x -2.32
	Overall	**y = 1.94x -2.54**
tail length	Forest 44	y = 1.83x -2.73
	Shaw	y = 1.52x -2.3
	Salamander	y = 1.24x -1.88
	Arthur Christ	y = 1.75x -2.63
	Beth's	y = 1.67x -2.52
	Mincke	y = 1.75x -2.59
	Overall	**y = 1.74x -2.6**
total length	Forest 44	y = 2.38x -4.21
	Shaw	y = 2.3x -4.04
	Salamander	y = 1.87x -3.28
	Arthur Christ	y = 2.21x -3.92
	Beth's	y = 1.96x -3.55
	Mincke	y = 2.11x -3.73
	Overall	**y = 2.23x -9.94**

Table S3: Length-mass regression equations for salamander morphology. Equations for regression lines expressing the relationship between mass with the length of salamander heads, bodies, tails, and the three body segments combined (total length). Both length and mass values were log-transformed in linear models used to calculate intercept and slope values for the regression lines.

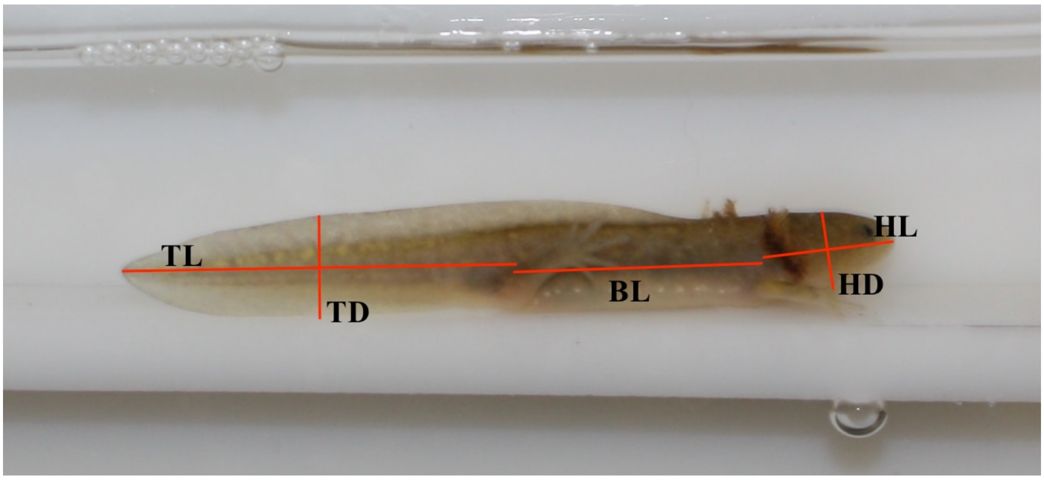
body segment	Factor	df	AIC	X^2	p
			mass		
	pond age	1	-452.73	0.00	0.949
	predator density	1	-452.29	0.45	0.505
			length		
head	pond age	1	1617.20	0.26	0.613
	predator density	1	1617.30	0.37	0.541
body	pond age	1	2245.60	0.05	0.816
	predator density	1	2245.60	0.04	0.841
tail	pond age	1	2732.60	0.10	0.757
	predator density	1	2732.50	0.01	0.909
total	pond age	1	3296.80	0.04	0.848
	predator density	1	3296.80	0.00	0.950
			mass:length co-variation		
head	pond age	**1**	**-588.61**	**12.21**	**0.002**
	predator density	1	-624.45	2.87	0.091
body	pond age	1	-1030.80	1.94	0.163
	predator density	**1**	**-1034.30**	**6.49**	**0.011**
tail	pond age	**1**	**-1073.50**	**5.43**	**0.020**
	predator density	1	-1078.30	3.44	0.064
total	pond age	1	-1296.80	0.13	0.717
	predator density	1	-1300.90	0.39	0.534
			shape		
head	**pond age**	**1**	**-1550.50**	**5.68**	**0.017**
	predator density	**1**	**-1549.80**	**6.40**	**0.011**
body	pond age	1	-1647.20	2.82	0.093
	predator density	1	-1649.50	0.55	0.459
tail	pond age	1	-1633.80	0.80	0.372
	predator density	1	-1634.30	0.26	0.612
overall	pond age	**1**	**-2052.60**	**5.53**	**0.019**
	predator density	1	-2056.30	1.78	0.182

Table S4. Influence of pond age and predator density on salamander morphology. Outputs of likelihood ratio tests of the influence of pond age and predator density on salamander morphological traits are reported. For mass-length co-variation, we tested the effects of pond age and predator density with separate models, to enable convergence of the complex models. Df = degrees of freedom, AIC = Akaike's Information Criterion. Cases where the factors significantly improved model fit to the data are highlighted in bold.

Invertebrate Species	Predatory (Y/N)	Beth's pond	Mincke pond	Salamander pond	Shaw pond	Arthur Christ pond	Forest 44 pond	total
Acilius fraternus	yes	0	0	1	0	0	0	1
Acilius sp. larvae	yes	0	3	0	0	0	0	3
Aeshna umbrosa	yes	0	0	1	0	0	0	1
Agabus sp. larvae	yes	2	1	0	0	0	0	3
Anaxyrus americanus	no	114	0	0	0	0	0	114
Chaoborus sp. larvae	no	108	38	0	0	0	0	146
Chauliodes sp. larvae	yes	0	0	5	0	0	0	5
Chironomid sp.	no	89	153	0	0	0	0	242
Enallagma sp.	no	1	0	0	0	1	0	2
Erythemis simplicollis	yes	0	0	0	0	5	0	5
Gyraulus parvus	no	0	3	0	0	0	0	3
Helisoma trivolvis	no	0	2	0	0	0	0	2
Helobdella sp.	no	0	7	0	0	0	0	7
Hesperocorixa sp.	no	0	0	1	0	0	0	1
Hydrobiomorpha sp.	no	0	0	1	0	0	0	1
Hyla versicolor	no	0	4	0	0	0	0	4
Laccophilus maculosa	no	1	2	0	0	0	1	4
Laccophilus sp. larvae	no	0	2	0	0	0	0	2
Libellula luctuosa	yes	7	0	0	0	0	0	7
Libellula pulchella	yes	4	0	0	0	0	0	4
Musculium transversum	no	0	200	0	0	0	0	200
Notonecta irrorata	yes	0	0	0	3	3	14	20
Notophthalamus viridescens louisianensis	yes	1	0	0	0	0	0	1
Ogliochaete	no	0	2	0	0	0	0	2
Pachydiplax longipennis	yes	5	0	0	2	15	0	22
Physa heterostropha	no	57	13	0	0	0	0	70
Pseudacris triseriata	no	0	4	0	0	0	0	4
Pseudosuccinea sp.	no	1	0	0	0	0	0	1
Rana clamitans	yes	3	0	0	25	0	0	28
Tropisternus blachelyi	yes	0	0	0	0	0	16	16

Tropisternus sp. 1	yes	0	0	0	0	0	1	1
Tropisternus sp. 4	yes	0	0	1	1	0	0	2
Tropisternus sp. larvae	no	0	0	0	0	0	3	3

Table S5: Counts of different species of invertebrates of focal ponds where salamanders were collected. Counts were performed within the same time period – July-August 2016 - as when salamander collected was executed, except for Beth's pond. Counts for Beth's pond come from a 2013 survey.



(b)

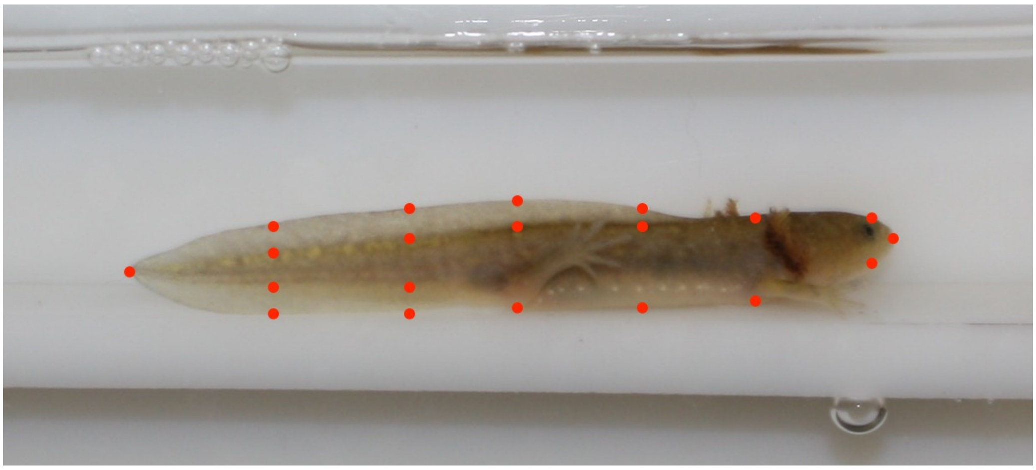


Fig. S1. Morphometric analysis of larval *A. maculatum* included both **(a)** measurements and (b) landmarks placed for shape analysis. Linear measurements included head length (HL), maximum head depth (HD), body length (BL), tail length (TL), and mid tail depth (TD). Geometric analysis involved placing twenty landmarks to outline the shape of, and included: tip of the snout (1) above the eye (2), below the eye (3), above vent (4), below vent (5), 50% length of body (6, 7, 8), in line with hind leg (9, 10, 11), at 25% length of tail (12, 13, 14, 15), at 50% length of tail (16, 17, 18, 19), and tip of tail (20).

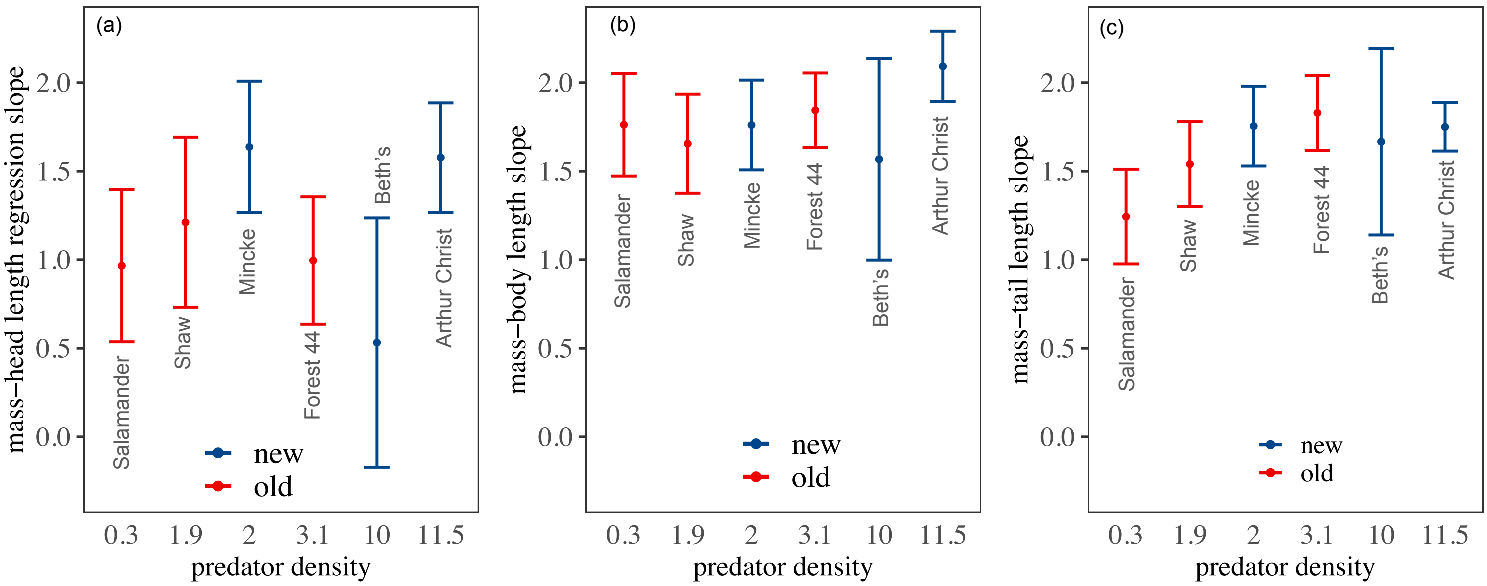


Fig. S2. The effect of pond age and predator density on mass-length relationships. The slope values for co-variation of salamander mass with (a) head length, (b) body length, and (c) tail length are displayed according to the predator density in ponds. Predators comprised invertebrates and adult newts. Red bars denote 'old' ponds that were constructed in the mid-1900s, and blue bars denote 'new' ponds that were constructed in 2008. Error bars denote 95% confidence intervals.

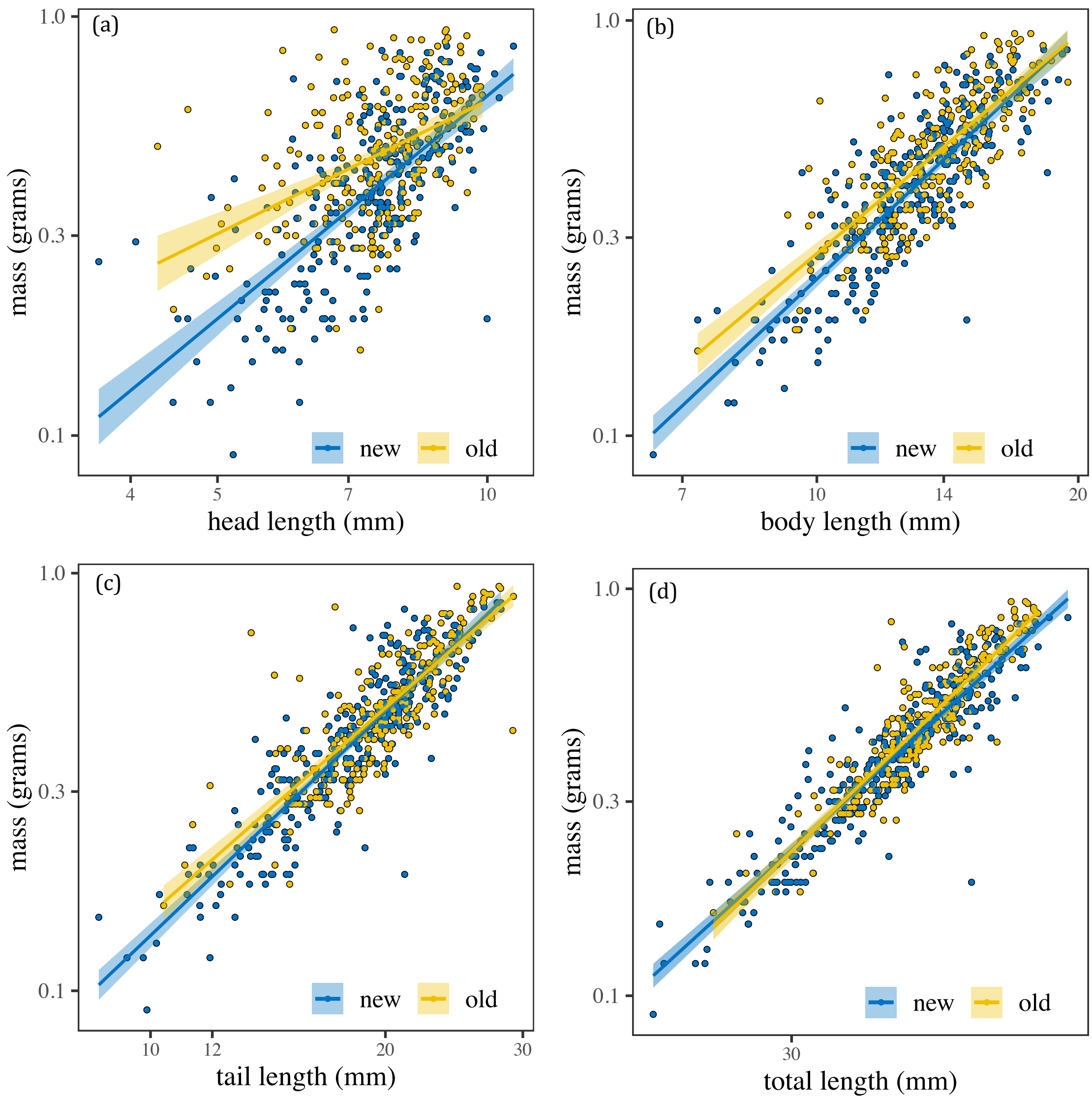


Fig. S3. The effect of pond age on mass-length relationships. Scaling of salamander mass with (a) head length, (b) body length, (c) tail length, and (d) total length are displayed, with regression lines drawn separately for new (blue) and old (yellow) ponds. New ponds were constructed in 2008. Old ponds were constructed in the mid-1900s. Shaded areas denote 95% confidence intervals.